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(54) Title: MONOCLONAL IgA ANTIBODY AGAINST RESPIRATORY SYNCYTIAL VIRUS

(57) Abstract

The present invention relates to antibodies directed against Respiratory Syncytial Virus (RSV), and hybridoma cell lines which produce them (e.g., HNK20). The antibodies can be used for the prevention or treatment of RSV infection and disease, as well as employed in methods for diagnosing RSV infection.

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MONOCLONAL IgA ANTIBODY AGAINST RESPIRATORY SYNCYTIAL VIRUS

Background of the Invention

This invention relates to monoclonal IgA antibodies to Respiratory Syncytial Virus and their use in therapeutic and diagnostic methods.

RSV appears in predictable yearly outbreaks. Annual outbreaks of lower respiratory tract disease in young children have been noted since at least the early 1940's (Adams, J. Pediatr. 20:405-420, 1941). RSV was implicated as the major cause of these outbreaks soon after its discovery in 1956 (Morris et al., Proc. Soc. Exp. Biol. Med. 92:544-549, 1956; Chanock et al., Am. J. Hyg. 66:281-290, 1957). RSV infects adults as well as infants, and causes serious lower respiratory tract disease primarily in very young infants, children with pulmonary or cardiac disease, the immunologically compromised and the elderly (MacIntosh et al., Virology, (Fields and Knipe eds.) 1045-1074, 1990). RSV infection is responsible for 40% to 50% of cases of children hospitalized with bronchiolitis and 25% of children with pneumonia (Heilman, J. Infect. Dis. 161:402-406, 1990). The number of cases requiring hospitalization in 1993 has been estimated at 91,000 with a cost of approximately $300,000,000 (Heilman, J. Infect. Dis. 161:402-406, 1990). Spread of the virus in hospitals is a particularly serious problem. When RSV infections are present in a hospital, 20% to 45% of infants may acquire a nosocomial RSV infection (Graman et al., Infect. Dis. Clin. N. Amer. 3:815, 1989). Premature infants and those hospitalized for cardiac or pulmonary diseases are thus placed at acute risk of developing lower respiratory tract disease. In a study of children with congenital heart disease, 21% of the RSV infections were acquired

nosocomially (Graman et al., Infect. Dis. Clin. N. Amer. 3:815, 1989).

To date, an effective vaccine against RSV has not been developed. In lieu of an active vaccine to protect high risk patients, especially infants, passive application of antibody may serve to protect these children during periods of known exposure. Intravenous treatment with immunoglobulin (IgG) containing anti-RSV activity is being tested in clinical trials (Hemming et al., Antimicrob. Agents Chemother. 31:1882-1886, 1987; Groothuis et al., Antimicrob. Agents Chemother. 35:1469-1473, 1991). While intravenous IgG might prevent lower respiratory tract disease, the evidence suggests that large doses and volumes of this material are required. Such treatment is not without potential adverse effects, including volume overload and circulatory failure.

In humans, upper airway infection generally precedes involvement of the lower respiratory tract (McIntosh et al., in Virology (Fields and Knipe eds.) 1045-1074, 1990). A study of modes of transmission shows that the virus is spread via fomites and self-inoculation of the nose or eyes, rather than by aerosol, suggesting that the infection does not initiate in the lower respiratory tract (Hall et al., J. Pediatr. 99:100-103, 1981). Viral infection is normally limited to the respiratory tract epithelium, and cell-to-cell spread is probably via secretions and cell-cell fusion (McIntosh et al., in Virology, (Fields and Knipe eds.) 1045-1074, 1990). Fused cells can be recovered from lung aspirates of infected patients (McIntosh et al., in Virology, (Fields and Knipe eds.) 1045-1074, 1990), but the importance of syncytium formation in pathogenesis or viral spread is not known.

None of the current approaches to prophylaxis of RSV focuses on the prevention of initial stages of

infection in the upper respiratory tract. Natural immunity in this compartment of the respiratory tract is mediated by IgA antibodies in the nasal secretions.

The immune response to RSV infection is short-lived. This allows repeated infection to occur in adults and children. In an adult challenge study, 40% of the subjects could be infected 3 times with the same challenge strain over a period of 26 months (Hall et al., J. Infect. Dis. 163:693-698, 1991). Up to 75% of children infected during their first season of RSV exposure are reinfected in their second season (Glezen et al., Am. J. Dis. Child. 140:543-546, 1986), although severe disease was uncommon after the initial infection. Circulating anti-RSV antibody can be protective when present in sufficient quantity, but its importance has been difficult to resolve. In animals, human IgG or specific monoclonal antibodies administered parenterally can protect against replication of the virus in the lung (Walsh et al., Infect. Immun. 43:756-758, 1984; Taylor et al., Immunology 52:137-142, 1984; Prince et al., Pediatr. Infect. Dis. 5:S201-S203 1986; Tempest et al., Biotechnology 9:266-271, 1991). High levels of circulating anti-RSV antibody protects primarily the lower respiratory tract (Walsh et al., Infect. Immun. 43:756-758, 1984). Moreover, the role of secretory antibody in protection against RSV has not been clearly established, but it appears that it may be an important mediator of the upper airway immunity. The titer of neutralizing antibody in nasal secretions correlates with decreased virus shedding and protection against disease in adult volunteers challenged with RSV (Mills et al., J. Immunol. 107:123-130, 1971; Watt et al., Vaccine 8:231-236, 1990). A decrease in viral shedding also correlates with the appearance of anti-RSV secretory IgA (sIgA) in nasal secretions of infants (McIntosh et al., J. Infect.

Dis. 138:24-32, 1978). However, not all of the neutralizing activity of nasal secretions is due to antibody (McIntosh et al., J. Infect. Dis. 138:24-32, 1978). A correlation between nasal anti-RSV antibody level and protection against infection or severe disease has not been demonstrated in human infants (Hall et al., J. Infect. Dis. 163:693-698, 1991; McIntosh et al., J. Infect. Dis. 138:24-32, 1978; Scott et al., J. Hyg. (Camb) 68:581-588, 1970; Bruhn et al., Am. J. Dis. Child. 131:145-148, 1977; Kaul et al., Am. J. Dis. Child. 135:1013-1016, 1981), but in animals, mucosal immunization protects against nasal infection (Reuman et al., J. Med. Virol. 32:67-72, 1990; Kanesaki et al., J. Virol. 65:657-63, 1991).

RSV is an enveloped, negative strand RNA virus belonging to the genus *Pneumovirus* of the *Paramyxoviridae* family (Fenner, Virology 71:371-378, 1975; Huang et al., J. Virol. 43, 1982). Two glycoproteins (90 kD and 68 kD) are exposed on the surface of the virion. The 90 kD heavily glycosylated G protein is responsible for binding of virus particles to target cells (Walsh et al., J. Gen. Virol. 65:761-767, 1984). The 68 kD F protein mediates fusion of the viral envelope with the cell membrane and syncytium formation (Walsh et al., J. Gen. Virol. 66:409-415, 1985). The F and G surface glycoproteins referred to above appear to be the primary protective antigens, with the nucleoprotein N and the envelope protein M2 having minor protective activity. Neutralizing and fusion-inhibiting monoclonal antibodies have been mapped to specific domains of F glycoprotein (Walsh et al., Infect. Immun. 43:756-758, 1984; Trudel et al., J. Gen. Virol. 68:2273-80, 1987; Beeler et al., J. Virol. 63:2941-50, 1989; Lopez et al., J. Virol. 64:927-30, 1990; Paradiso et al., Vaccine 9:231-7, 1991). Monoclonal antibodies against the G glycoprotein are less

likely to neutralize virus than those against the F glycoprotein and do not have fusion inhibiting activity (Norrby et al., Proc. Natl. Acad. Sci. USA 84:6572-6576, 1987; Garcia-Barreno et al., J. Virol. 63:925-932, 1989; Walsh et al., J. Gen. Virol. 70:2953-2961, 1989). The amino acid sequence of F glycoprotein is approximately 90% conserved between the RSV subgroups responsible for human infection (Toms, FEMS Microbiol. Immunol. 76:243-256, 1991). Conserved epitopes include some that mediate neutralization and fusion inhibition (Tempest et al., Biotechnology 9:266-271, 1991; Toms, FEMS Microbiol. Immunol. 76:243-256, 1991). The G glycoprotein which is primarily responsible for differences between subgroups A and B is only 53% conserved between the two subgroups (Johnson et al., Proc. Natl. Acad. Sci. USA 84:5625-5629, 1987). Immunization with a recombinant vaccinia virus expressing N or M2 induces a minor protective response in mice (King et al., J. Virol. 61:2885-2890, 1987; Connors et al., J. Virol. 65:1634-7, 1991). This response may be due primarily to CTL activity, since anti-N monoclonal antibody does not protect when passively administered to mice (Taylor et al., Immunology 52:137-142, 1984). Moreover, N has been shown to be a CTL target in mice and humans (King et al., J. Virol. 61:2885-2890, 1987).

An early vaccine consisting of formalin-inactivated alum-adsorbed RSV elicited neutralizing and complement-fixing serum antibody in a clinical trial. However, vaccinated children were not protected and had more severe lower respiratory tract disease upon subsequent natural infection (Kapikian et al., Am. J. Epidemiol. 89:405-421, 1969). The reason for the enhanced disease has not been fully explained. Cotton rats immunized with formalin-inactivated RSV developed a similar pathological response (Prince et al., J. Virol.

57:721-728, 1986), providing a method of testing the safety of new vaccines.

Efforts have focused in the past on developing attenuated live virus vaccines. To date, those vaccines have been found to be ineffective (Belshe et al., J. Infect. Dis. 145:311-319, 1982), insufficiently attenuated (Wright, J. Pediatr. 88:931-939, 1976), or genetically unstable (McIntosh et al., Pediatr. Res. 8:689-696, 1974; Hodes et al., Proc. Soc. Exp. Biol. Med. 145:1158-1164, 1974). More recent efforts have focused on the RSV surface glycoproteins F and G. Immunization with purified F glycoprotein have shown to be effective in cotton rats and is currently in clinical trials (Walsh, J. Infect. Dis. 155:1198-1204, 1987; Routledge, J. Gen. Virol. 69:293-303, 1988; Murphy, Vaccine 8:496-502, 1990). However, some preparations of F glycoprotein have been shown to cause enhanced lung pathology upon subsequent RSV infection in cotton rats (Murphy, Vaccine 8:497-502, 1990). Recombinant chimeric FG glycoprotein produced in a baculovirus expression system elicits a protective immune response in cotton rats when given parenterally (Brideau et al., J. Virol. 70:2637-44, 1989). As with F glycoprotein alone, the FG vaccine was also shown to cause some enhanced pulmonary pathology in cotton rats (Wathen et al., J. Infect. Dis. 163:477-82, 1991; Connors et al., Vaccine 10:475-484, 1992). Recombinant vaccinia viruses expressing F, G, or M2 envelope protein, or the nucleoprotein N, have been tested in several animal models. F and G recombinants have shown the most promise, inducing protective immunity in mice (Scott et al., J. Virol. 60:607-613, 1986; Olmsted et al., Proc. Natl. Acad. Sci. USA 83:7462-7466, 1986), cotton rats (Elango et al., Proc. Natl. Acad. Sci. USA 83:1906-1910, 1986), and owl monkeys (Olmsted et al., Vaccine 6:519-524, 1988). The response in chimpanzees

however was markedly lower (Collins et al., Vaccine 8:164-8, 1990). Adenovirus is also being examined as a vector for expression of RSV F glycoprotein (Hsu et al., Vaccines 91, 1991).

A need exists, therefore, for effective approaches to the prevention of RSV disease. The present invention seeks to fill that need.

Summary of the Invention

According to one aspect, the invention provides a neutralizing monoclonal IgA antibody to Respiratory Syncytial Virus (RSV), e.g., HNK20, that is directed against, e.g., the F glycoprotein of RSV. Preferably, the monoclonal antibodies are in substantially pure form, free from other immunological material.

According to another aspect, the invention provides a composition containing one or more of the monoclonal IgA antibodies and a suitable carrier or diluent.

The invention further provides a method of treating or preventing RSV infection in a host involving administering to the host an amount of antibody of the invention sufficient to achieve the treatment or prevention of disease. The antibody may be administered parenterally to the host, e.g. intravenously, or may be administered to a mucosal surface of the host. A preferred mode of administration is intranasal.

In another aspect, the invention features the use of a monoclonal IgA antibody to Respiratory Syncytial Virus for the preparation of a medicament for the treatment or prevention of Respiratory Syncytial Virus infection in a host.

The invention also provides pharmaceutical compositions suitable for treatment or prevention of RSV infection containing an effective amount of the antibody

of the invention and a pharmaceutically acceptable carrier or diluent.

The present invention also provides a process for producing monoclonal IgA antibodies to RSV (e.g., HNK20) in which the hybridoma cell line expressing it (e.g., HNK20) is cultured, and the antibodies so produced are recovered. The process is preferably carried out by culturing the cell line *in vitro* in a nutrient culture medium and recovering the antibodies from the culture supernatant.

The invention also provides a method of diagnosing the presence of RSV antigen in a biological sample, in which the antigen is contacted with monoclonal IgA antibodies of the invention (e.g, HNK20) and the presence of the antigen is detected by an immunoassay, e.g., immunofluorescent microscopy, immuno-electron microscopy, solid-phase radiometric assay, or enzyme-linked immunoassay (ELISA). The method is preferably carried out by incubating a sample taken from a human or animal with the antibody bound to a solid support, washing the solid support, and incubating it with radiolabeled or enzyme-labeled antibody as tracer. The sample may be, e.g., nasal secretions, serum, nasal washings, pharyngeal secretions, or bronchial secretions.

The invention further provides a method of isolating RSV antigens from a biological sample involving contacting the sample with the antibody of the invention, the antibody being bound to a solid support, in order to cause RSV antigen to bind to the antibody, and subsequently separating the RSV antigen from the solid support.

The invention further provides a kit comprising a first container containing a plastic substrate coated with the antibody of the invention and a second container containing antibodies of the invention to which antibody

a radio-label or enzyme label has been attached. Preferably, the plastic substrate is polystyrene in the form of beads, sticks, or tubes.

According to a further aspect of the invention, a hybridoma cell line (e.g., HNK20) which secretes monoclonal antibodies to RSV antigen is provided. The cell line is preferably in substantially pure form free from other cellular material.

Compositions containing the cell line are also provided in the invention, containing the cell line together with a nutrient medium capable of maintaining the cell line. An appropriate medium contains a source of carbon, a source of nitrogen and, if desired, vitamins and/or organic salts.

According to a further aspect of the invention, a process for propagating the hybridoma cell line of the invention (e.g., HNK20) is provided, and involves culturing the cells in a nutrient culturing medium. The method of propagation also represents a means of producing the antibodies of the invention which may be separated from the culture medium. Preferably, the propagation of the hybridoma cell line of the invention is carried out *in vitro*, wherein the cell line is cultured in a nutrient culture medium. An appropriate nutrient culture medium for the cells of the present invention contains a source of carbon, a source of nitrogen and if desired vitamins and/or inorganic salts. For example, RPMI 1640 medium supplemented with 10% fetal bovine serum may be used. Another suitable nutrient medium is Sigma Serum-free and Protein-free Hybridoma medium.

An important advantage of the antibody of the invention is that it can reduce infection of the upper airways, where IgA is the major effector antibody isotype. Particularly good results are obtained when the

antibody is administered intranasally. This is one feature which distinguishes the antibody of the present invention from other monoclonal antibodies or immune globulin preparations which are administered parentally (via intravenous or intramuscular routes) to reduce infection of the lower respiratory tract, while allowing upper respiratory tract infection to occur. The monoclonal antibody of the present invention is particularly useful for passive treatment and protection of hospitalized patients, especially infants, from RSV while at the same time limiting viral spread during outbreaks.

As noted earlier, administration by the intranasal route is preferred over the parenteral route in that the intranasal route has the advantage of greater safety. Adverse reactions of the allergic type to topical (intranasal) antibody are localized to the nose or upper airways rather than being systemic hypersensitivity reactions, that can have severe consequences for the host. Parenteral administration of immunoglobulin (IVIG) or monoclonal antibodies may result in anti-idiotypic antibody responses with potential adverse effects upon the recipient. The concentration of intranasal antibody required for protection is significantly lower (200 times less) than that required for parenteral antibody. Small infants may not tolerate the large amounts (volumes) of IVIG required for protection. Finally, IgA applied topically may have advantages over IgG in being polyvalent rather than monovalent, and hence more efficacious in binding or neutralizing virus. Moreover, IgA binds complement to a very limited degree as compared to IgG, with the result that IgA is less likely to participate in inflammatory reactions that could cause side effects in the treated individual.

Detailed Description

The drawings are first described.

Drawings

Fig. 1 is polyacrylamide gel electrophoresis (SDS-PAGE) analysis of anti-RSV HNK monoclonal antibodies from hybridoma cells grown in protein-free medium.

Fig. 2 is a graph showing the effect of increasing doses of HNK20 given intranasally to mice 1 hour prior to RSV challenge (significance by unpaired t-test: 0.1 μg, p = .03; 1 μg, p = .001; 10 μg, p = .001; 100 μg, p = .001).

Fig. 3 is a graph showing the result of intranasal treatment of mice with monoclonal IgA (HNK20 and 2D6) 1 hour prior to RSV challenge (significance by unpaired t-test: lungs, p = .02; nasal turbinates, p = .01).

Fig. 4 is a graph showing the result of intranasal treatment of mice with HNK20 1 to 3 days prior to RSV challenge (significance by unpaired t-test: 24 hours, p = .02; 48 hours, p = .03; 72 hours, p = .03).

Fig. 5 is SDS-PAGE analysis of ^{35}S-labelled RSV-infected lysates immunoprecipitated with monoclonal antibodies generated from cell lines HNK18, HNK20, 133/1H (anti-F), and 2D6 (anti-*V. cholerae*).

Fig. 6 is an immunoblot of HNK20 IgA run on a non-reducing 5-15% gradient SDS-PAGE gel (the gel was blotted on to nitrocellulose and reacted with alkaline phosphatase-labelled rabbit anti-mouse IgA-alpha chain specific antibodies).

Hybridoma cell lines were prepared by immunizing BALB/c mice with live RSV delivered intranasally or intragastrically. Mucosal immunization was carried out in order to elicit preferentially a mucosal IgA response. Four days after the final immunization, the mice were sacrificed and lung and Peyer's patch leukocytes were

isolated and separately fused with P3U1 myeloma cells. The resulting hybridomas were screened for anti-RSV antibody production by ELISA. Lung cell fusions yielded 24 hybridomas secreting anti-RSV antibody. The fusions were carried out according to the methods of Kohler et al. (Eur. J. Immunol. 6:292-295, 1976). Eight of these antibodies were of the IgA subtype. No anti-RSV hybridomas were obtained from the Peyer's patch fusions.

After their identification and cloning, anti-RSV monoclonal antibodies were tested for recognition of RSV subgroups A and B. Binding to RSV subgroup A (strains A2 and Long) and subgroup B (strain 18537) was compared by ELISA. The results are set forth in Table 1 below.

Table 1. Binding of mAbs to RSV strains A2 (subgroup A), Long (subgroup A) and 18537 (subgroup B)

Experiment R0265A

	OD_{405}		
mAb	A2(A)	Long(A)	18357(B)
HNK 4	0.214	0.162	0.009
HNK 10	0.054	0.044	0.053
HNK 11	0.141	0.123	0.146
HNK 12	0.031	0.021	0.056
HNK 13	0.026	0.004	0.009
HNK 16	0.540	0.598	0.579
HNK 17	0.176	0.176	0.066
HNK 18	0.356	0.423	0.374
HNK 19	0.151	0.204	0.137

Experiment R0298A

	OD_{405}		
mAb	A2(A)	Long(A)	18357(B)
HNK 20	0.191	0.227	0.146
HNK 21	0.250	0.295	0.248
HNK 22	0.122	0.205	0.062
HNK 23	0.081	0.186	0.026
HNK 24	0.230	0.258	0.203

Five of eight IgAs (HNK 11, 19, 20, 22, and 24) were found to bind to all three strains. These five monoclonal antibodies, along with three subgroup cross-

reactive IgG_{2a} monoclonal antibodies (HNK 16, 18 and 21), were selected for further study. The eight cross-reactive hybridomas were adapted to growth in protein-free culture medium. The culture medium was collected, concentrated, and assayed for antibody concentration. Fig. 1 shows a SDS-PAGE analysis of each concentrated antibody preparation. The gel was stained with Coomassie blue in order to visualize the proteins.

The above anti-RSV monoclonal antibodies were tested for *in vitro* neutralization by a plaque-reduction assay using strain A2 (see Table 2). Two antibodies, HNK20 and HNK24, showed neutralizing activity. HNK20 was the most effective, giving a 50% reduction in plaque numbers at a concentration of 0.1 μg/ml or less. The 50% effective dose of HNK20 was the same for neutralization of 18537, a subgroup B strain.

Table 2. *In vitro* neutralization of RSV

MAb	Isotype	Neutralization[a]
HNK11	IgA	>100 μg/ml
HNK19	IgA	>100 μg/ml
HNK20	IgA	0.1 μg/ml
HNK22	IgA	>100 μg/ml
HNK24	IgA	10 μg/ml
HNK16	IgG2a	>100 μg/ml
HNK18	IgG2a	>100 μg/ml
HNK21	IgG2a	>100 μg/ml

[a] lowest antibody concentration giving 50% plaque reduction.

Table 2 shows that none of the IgG monoclonal antibodies neutralizes the virus.

The above-described eight anti-RSV monoclonal antibodies have been screened for protection against pulmonary RSV infection in the mouse model. Mice were challenged intranasally with approximately 10^6 plaque forming units (PFU) of virus 24 hours after an intranasal dose of anti-RSV or non-specific control monoclonal antibody (2D6 - an IgA against *Vibrio cholerae*). Four

days later, the lungs were removed and homogenized, and the virus content of the lung tissue determined. The results are set forth in Table 3.

Table 3. *In vivo* protection experiments: Reduction in lung virus titer after intranasal treatment with monoclonal antibody 24 hours before RSV challenge.

Experiment number	Treatment	Lung PFU/g($\times 10^5$)
1	2D6	1.8±0.1
	HNK16(IgG)	1.4±0.4
	HNK18(IgG)	0.3±0.1
	HNK21(IgG)	0.7±0.1
2	2D6	0.9±0.2
	HNK11(IgA)	1.0±0.3
	HNK19(IgA)	0.6±0.2
	HNK22(IgA)	0.7±0.2
3	2D6	1.1±0.3
	HNK20(IgA)	0.02±0.02
4	2D6	0.7±0.4
	HNK24(IgA)	0.3±0.1

Treatment with monoclonal antibodies HNK18 (an IgG antibody) and HNK20 (an IgA antibody) resulted in the reduction in lung viral titer of approximately 1 log or greater.

The antibody HNK20 has been tested over a range of concentrations for its ability to protect against infection of mouse lungs. The results are set forth in Fig. 2. HNK20 antibody was administered intranasally 1 hour prior to viral challenge. Maximum protection was seen at a dose of between 1 and 10 μg per mouse.

The HNK20 monoclonal antibody protects against replication of RSV in nasal mucosa. This is demonstrated by the data set forth in Fig. 3. Mice were challenged as described above, and PFU/g nasal turbinate tissue was determined four days after challenge. HNK20 or 2D6 monoclonal IgA was administered 1 hour before viral

challenge. HNK20 produced a greater than 1 log decrease in viral PFU in nasal tissue.

Protection of the lungs of mice from infection was similar whether HNK20 antibody was given 1 hour or 24 hours before viral challenge. Fig. 4 shows the effect on lung protection in mice when HNK20 is administered intranasally 24, 48, and 72 hours prior to challenge. Protection is seen at all three time points, with the mice treated 24 hours before challenge being better protected than those treated at 48 and 72 hours.

HNK20 was also tested for protective activity in cotton rats, a well established model for RSV infection of the respiratory tract. HNK20 was administered intranasally 1 hour, 3 hours, and 6 hours prior to RSV challenge. Cotton rats were sacrificed 4 days after infection and titers of RSV in the lung homogenates and nasal washes were assessed. Significant protection of both lung and nasal tissue was observed at all three points (see Table 4). Many of the HNK20-treated cotton rats, particularly at the 1 hour time point, had no recoverable virus.

TABLE 4

1. Nasal wash titers on day +4

Group	Treatment (mg/kg)	Individual RSV titers(log10/0.05 ml) 1	2	3	4	Mean	S.D.	No. infec. No./grp
1	Placebo	2.8	3.3	2.8	3.3	3.05	0.29	4/4
2	HulSG	0	0	0	0	0.00	0.00	0/4
3	IgA - 1h	0	0	0	0	0.00	0.00	0/4
4	IgA - 3h	0	0	0	1.6	0.45	0.90	1/4
5	iGA - 6h	1.8	1.8	0	0	0.90	1.04	2/4

0=undetected (less than the minimal detectable titer [<1.3 log10/0.5 ml])
HulSG=human immune serum globulin (Armour Pharmaceuticals)
IgA=OraVax IgA monoclonal antibody (Mab)

1. RSV titers in the lung on day +4

Group	Treatment (mg/kg)	Individual RSV titers(log10/g lung) 1	2	3	4	Mean	S.D.	No. infec. No./grp
1	Placebo	3.3	2.8	2.8	2.8	2.93	0.25	4/4
2	HulSG	0	0	2.3	0	0.58	1.15	1/4
3	IgA - 1h	0	2.3	0	0	0.58	1.15	1/4
4	IgA - 3h	2.6	0	2.3	0	1.28	1.49	2/4
5	IgA - 6h	0	2.3	0	3.3	1.40	1.67	2/4

0= undetected (less than the minimal detectable titer [<1.3 log 10/g lung])
HulSG=human immune serum globulin (Armour Pharmaceuticals)
IgA-OraVax IgA monoclonal antibody (Mab)

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The protein specificities of HNK18 and HNK20 have been examined by immunoprecipitation of radiolabeled lysates from RSV-infected VERO cells. HNK20 precipitated a pair of bands corresponding in mobility to the F_1 and F_2 subunits of the F glycoprotein. This is shown in Fig. 5. These bands co-migrated with the bands precipitated by 133/1H, which is a monoclonal antibody previously shown to bind to F glycoprotein. HNK18 precipitated a protein with a molecular weight of approximately 47 kD. The identity of this band has not yet been determined, but its molecular weight is close to that of N protein (43.5 kD). Further evidence for F glycoprotein specificity of HNK20 was provided by an ELISA in which the monoclonal antibody was shown to bind to VERO cells infected with a recombinant vaccinia virus expressing RSV F glycoprotein. This is shown in Table 5. Binding to uninfected VERO cells or VERO cells infected with recombinant viruses expressing glycoprotein G or hepatitis VP59 was negligible.

Table 5. Binding of mAbs to VERO cells infected with recombinant vaccinia viruses expressing RSV F, RSV G, or hepatitis VP 59

	OD_{405}			
mAb	uninfected	vac/VP59	vac/F	vac/G
HNK20	0.011	0.000	0.137	0.003
133/1H (anti-F)*	0.189	0.174	0.453	0.218
131/2G(anti-G)*	0.191	0.176	0.193	0.317

* monoclonal antibodies against RSV (obtained from Biodesign International)

Structurally, HNK20 has been shown to possess κ light chains by ELISA using light chain-specific antibodies. The polymeric structure of HNK20 was examined by fractionating the monoclonal antibody on a 5 to 15% gradient acrylamide gel under non-reducing conditions and staining for IgA bands in an immunoblot. This is shown in Fig. 6. The antibody is shown to be

produced in monomeric, dimeric, and higher polymeric forms. The major species was dimeric.

Studies have shown that HNK20 antibody exhibits good storage properties under varying conditions. Thus, HNK20 antibody stored under various conditions for 1 to 2 months was examined by RSV-binding ELISA and gel electrophoresis under reducing and non-reducing conditions. The antibody proved to be very stable at 4°C, -80°C, or -20°C in glycerol. A slight reduction in ELISA reactivity and the appearance of lower molecular weight bands was found in the -80°C and -20°C samples at a two-month time point.

The cell line HNK20 secreting monoclonal antibody HNK20 mAb was been deposited with The American Type Culture Collection (ATCC, 12301 Parklawn Drive, Rockville, Maryland 20852, USA) on July 1, 1993, and has been accorded the accession number ATCC HB 11394.

The monoclonal antibody secreted by hybridoma HNK20 may be recovered from mouse ascites fluid. This process of both propagating the cell lines and producing the antibodies represents a further aspect of this invention. This means of obtaining the monoclonal antibodies of the invention offers the advantage that the yield may, for example, be as much as 10-fold higher than the yield obtained from the bulk culture of the cells. The period of time normally taken to carry out this method is about 2 to 3 weeks.

The monoclonal antibody secreted by the HNK20 hybridoma may be also produced at high concentration and purity by culture of the hybridoma cells in a hollow fiber bioreactor such as the Maximizer 1000 produced by Endotronics, Inc. This means of obtaining the monoclonal antibodies of the invention offers the advantages of high yield (approximately 1 mg/ml) and greater purity than antibodies produced in mouse ascites.

The antibodies of the invention may be used unpurified from the sources described above. However, preferably the antibodies are subjected to purification before use. For example, IgA monoclonal antibodies may be purified from cell culture fluid by sequential anion exchange chromatography on Q Sepharose followed by gel filtration using Sephacryl S-300, as described previously (Soman et al., J. Immunol. 150:116A, 1993).

The monoclonal antibodies of the invention, e.g., those produced by the cell line HNK20, have particular utility in passive treatment of patients suffering from RSV infection or exposed to RSV. It is known that natural hosts for RSV are humans, chimpanzees, and cattle. In addition, non-human primates, including cebus and owl monkeys, develop clinical disease when infected with RSV. Moreover, RSV replicates in the upper respiratory tract of adult ferrets and in the lungs of lambs. The vast majority of recent studies have used cotton rats or mice as models to study RSV pathogenesis and protection against RSV infection. RSV replicates in the upper and lower respiratory tracts of these species, with peak viral titers appearing after 4 or 5 days. These rodents are particularly suitable for immunological studies aimed at demonstrating protection against RSV. Studies of passive immunization with IgG have shown that the results obtained from rodents are predictive of the activity of IgG in monkeys and humans. The results obtained in mouse and cotton rat models for the present IgA monoclonal antibody are therefore believed to be predictive of probable efficacy in humans, including human infants.

The monoclonal antibodies can be used, according to one aspect of the invention, for the passive treatment or prevention of RSV infection in a host, including humans. The method comprises administering to the

patient an effective amount of HNK20. Typically, the antibody is administered to a mucosal surface, and may be administered orally or intranasally. The amount of antibody which is administered will vary from 50 μg/kg to 5 mg/kg body weight.

For therapeutic and/or preventative use, the antibody compositions of the invention may be in solid or liquid form with a suitable pharmaceutical carrier and/or a diluent known in the art. The compositions are prepared in a conventional manner and comprise an effective amount of the antibody, typically 50 μg/kg to 5 mg/kg body weight.

The compositions may be in the form of an injectable solution, or in the form of solutions, suspensions, or powder. Preferably, the composition is formulated for nasal delivery to provide protection of the upper respiratory tract, or for aerosol delivery to provide protection of the lower respiratory tract.

The antibodies produced by the cell lines of the invention, e.g., HNK20, also have utility in providing accurate screening tests for patients infected by RSV. The antibodies can be used in assay systems employing, for example, immunofluorescent microscopy, or immuno-electron microscopy, for detecting the presence of RSV in cellular material or cellular secretions. Quantitative assays may be carried out by solid phase radiometric assays or ELISA.

A method of diagnosing the presence of RSV antigen in a biological sample according to the invention includes contacting an antigen with monoclonal IgA antibodies to RSV, e.g., those secreted by hybridoma cell line HNK20, and detecting the presence of the RSV antigen by immunofluorescent microscopy, immuno-electron microscopy, in a solid-phase radiometric assay system, or in an enzyme-linked immunoassay. Preferably, a sample

taken from a human or animal is incubated with the IgA antibody (e.g., HNK20) in solid phase, followed by washing and incubating with radiolabeled or enzyme-labeled IgA antibody as tracer. In an assay system, the preferred solid phase comprises a plastic or glass substrate on which the antibodies are coated. A particularly preferred substrate is in the form of beads, sticks, tubes, or plates, made of, e.g., example polystyrene.

The present invention also provides these coated substrates, e.g. beads, in diagnostic kits. The kits comprise a first container which contains a plastic or glass substrate (such as beads, sticks, plates, or tubes) coated with IgA antibodies to RSV (e.g., HNK20), and the second container containing IgA antibodies to RSV (e.g., HNK20) to which a radiolabel has been attached.

Instead of employing a radiolabel, it is possible as an alternative assay method in accordance with the invention, to employ, for example, an enzyme-label or a biotin-label which will generally be linked to the IgA antibody to RSV. Diagnosis of RSV infection is achieved by reacting a clinical specimen (containing RSV) with the substrate coated with the IgA antibodies to RSV. After a suitable incubation period, the second (radio- or enzyme-labeled) antibody is added (followed by substrate in the case of enzyme-labeled antibody). The reaction measures the presence and amount of RSV antigen in the clinical sample.

When coupled to a solid phase such as bromide activated Sepharose, the monoclonal antibodies of the present invention can also be used to remove RSV from human or animal biological material, either for the purpose of preparing RSV in a purified form for use in preparing vaccines, or for the removal of RSV from biological material to be administered to patients. The

present invention therefore provides a method of isolating RSV antigen from a biological sample with IgA antibodies to RSV in the solid phase to cause binding to the antigen to the antibody and subsequently separating the desired purified material from the solid phase.

The HNK20 hybridoma can be used to construct novel antibodies containing mouse and human sequences. IgA heavy and light chain variable domains from an IgA antibody against RSV (e.g., HNK20) are combined with human immunoglobulin heavy and light chain constant domains using recombinant DNA techniques to yield antibodies with predominantly human sequence and the binding specificity of the mouse monoclonal IgA antibodies against RSV (e.g., HNK20). Such antibodies, termed chimeric or humanized antibodies, are particularly useful for parenteral treatment of humans, as they are less likely to provoke an immune or allergic response to the antibody.

The monoclonal IgA antibodies of the invention can be bound to secretory component to yield complexes with increased resistance to digestion by proteolytic enzymes. Secretory component is combined with polymeric IgA in one of several ways. In one method, IgA antibodies and secretory component are mixed in solution and allowed to associate. In another method, the IgA-secreting hybridoma cells are transfected with an expression vector containing the cDNA for secretory component. The resulting cells produce IgA-secretory component complexes. In a third method, a cultured epithelial cell line, such as MDCK, is transfected with an expression vector containing the cDNA for polymeric immunoglobulin receptor. The transfected epithelial cells are grown on porous membrane filters in chambers in which the medium bathing the apical and basolateral sides of the cells are separate. Polymeric immunoglobulin receptors effect

transport of IgA from the basolateral medium to the apical medium. Polymeric IgA is added to the basolateral medium and IgA is released into the apical medium in association with the secretory component, a cleaved portion of the polymeric immunoglobulin receptor.

EXAMPLES

The invention will now be further illustrated by the following non-limiting examples.

Example 1

Three mice are infected intranasally with 10^6 PFU RSV in a volume of 25 μl, while under isoflurane anesthesia. After 4 days, nasal turbinates are removed and a pooled 10% homogenate of nasal tissue in tissue culture medium is prepared. The sample is titrated by plaque assay, diluted to 10^6 PFU/25 μl, and inoculated into another group of 3 mice. Continued passages are performed. After adaptation to mice, a significant increase in lung virus titers is observed. The virus levels are tested at days 3, 4, 5, and 6 after inoculation to determine the peak of viral replication. The optimal conditions defined by these experiments are used for all subsequent mouse challenge experiments.

Example 2

The amount of antibody to RSV, e.g., HNK20, required for protection is tested by applying different amounts of monoclonal antibody intranasally one or more hours before viral challenge. Amounts ranging from 0.1 to 100 μg per mouse are given intranasally in a volume of 25 μl while the mice are under isoflurane anesthesia.

To carry out animal experiments, approximately 50 mg of monoclonal IgA antibody is produced. The antibodies are partially purified and processed to yield monomeric and polymeric fractions of IgA antibody against RSV (e.g., HNK20). The hybridoma (e.g., HNK20) is cloned

3 times, and adapted to growth in protein-free medium (Sigma Chemical Company). The hybridoma is grown in four 500 ml spinner flasks, allowing 2 L of spent medium to be collected every 2 days. A total of 8 L is collected. The medium is concentrated approximately 200 fold in a stirred cell with 100 kD cut-off membrane. The resulting crude concentrate contains about 50% pure monoclonal antibody at about 2-5 mg/ml.

The monoclonal antibody IgA (e.g., HNK20) is passed over a DEAE-sepharose column and eluted with 0.3 M sodium chloride. This material is greater than 90% IgA, and is separated into monomeric, polymeric, and aggregate fractions by Sephacryl S300 size exclusion chromatography. Molecular weight determinations are made by HPLC analysis and SDS-PAGE using a 5 to 15% gradient gel.

Example 3

The length of protection afforded by monoclonal IgA antibodies to RSV (e.g., HNK20) monoclonal IgA is examined when the antibody is delivered to a viscous or bioadhesive carrier. Carriers to be tested include methylcellulose and neutralized polyacrylic acid. A solution of 50 μg/ml antibody in 0.25% methylcellulose, or 50 μg/ml antibody in 1.5% polyacrylic acid, is prepared. Mice are treated by intranasal instillation of 10 μl of antibody-carrier mixture. Control mice receive non-specific monoclonal IgA 2D6 in saline, 2D6 mixed with carriers, or monoclonal IgA antibody against RSV (e.g., HNK20) in saline. Different groups of mice are challenged intranasally with RSV at 1 hour, 5 hours, 10 hours or 15 hours after treatment. Four days after challenge, mice are sacrificed and RSV titers in nasal tissues are determined. Carriers that extend the length of protection are examined further for effects on doses required for protection and adverse effects.

CLAIMS:

1. A monoclonal IgA antibody to Respiratory Syncytial Virus.

2. A composition comprising the monoclonal IgA antibody of claim 1 and a carrier or diluent.

3. Use of a monoclonal IgA antibody to Respiratory Syncytial Virus for the preparation of a medicament for the treatment or prevention of Respiratory Syncytial Virus infection in a host.

4. A method of detecting the presence of a Respiratory Syncytial Virus antigen in a biological sample, said method comprising the steps of:

(a) contacting said sample with a monoclonal IgA antibody to Respiratory Syncytial Virus; and

(b) detecting the presence of said antigen in said sample by an immunoassay.

5. The method of claim 4, wherein said immunoassay is immunofluorescent microscopy, immuno-electron microscopy, a solid-phase radiometric assay, or an enzyme-linked immunoassay.

6. A method of isolating a Respiratory Syncytial Virus antigen from a biological sample comprising the steps of:

(a) contacting said sample with an antibody to Respiratory Syncytial Virus, said antibody being bound to a solid support; and

(b) separating said antigen from said solid phase.

7. A kit comprising:

(a) a first container containing a plastic substrate coated with an antibody to Respiratory Syncytial Virus; and

(b) a second container containing an antibody to Respiratory Syncytial Virus, to which antibody a radio-label or enzyme label has been attached.

8. The kit of claim 7, in which the plastic substrate is polystyrene in the forms of beads, sticks, tubes, or plates.

9. A hybridoma cell line which produces a monoclonal IgA antibody to Respiratory Syncytial Virus.

10. A composition comprising a hybridoma cell line which produces a monoclonal IgA antibody to Respiratory Syncytial Virus and a nutrient medium capable of maintaining the cell line.

11. The monoclonal IgA antibody of claim 1, wherein said antibody is produced by hybridoma cell line HNK20.

12. The use of claim 3, wherein said monoclonal IgA antibody is produced by hybridoma cell line HNK20.

13. The method of claim 4, wherein said monoclonal IgA antibody is produced by hybridoma cell line HNK20.

14. The method of claim 6, wherein said monoclonal IgA antibody is produced by hybridoma cell line HNK20.

15. The kit of claim 7, wherein said monoclonal IgA antibody is produced by hybridoma cell line HNK20.

16. The hybridoma cell line of claim 9, wherein said hybridoma cell line is HNK20.

17. The composition of claim 10, wherein said hybridoma cell line is HNK20.

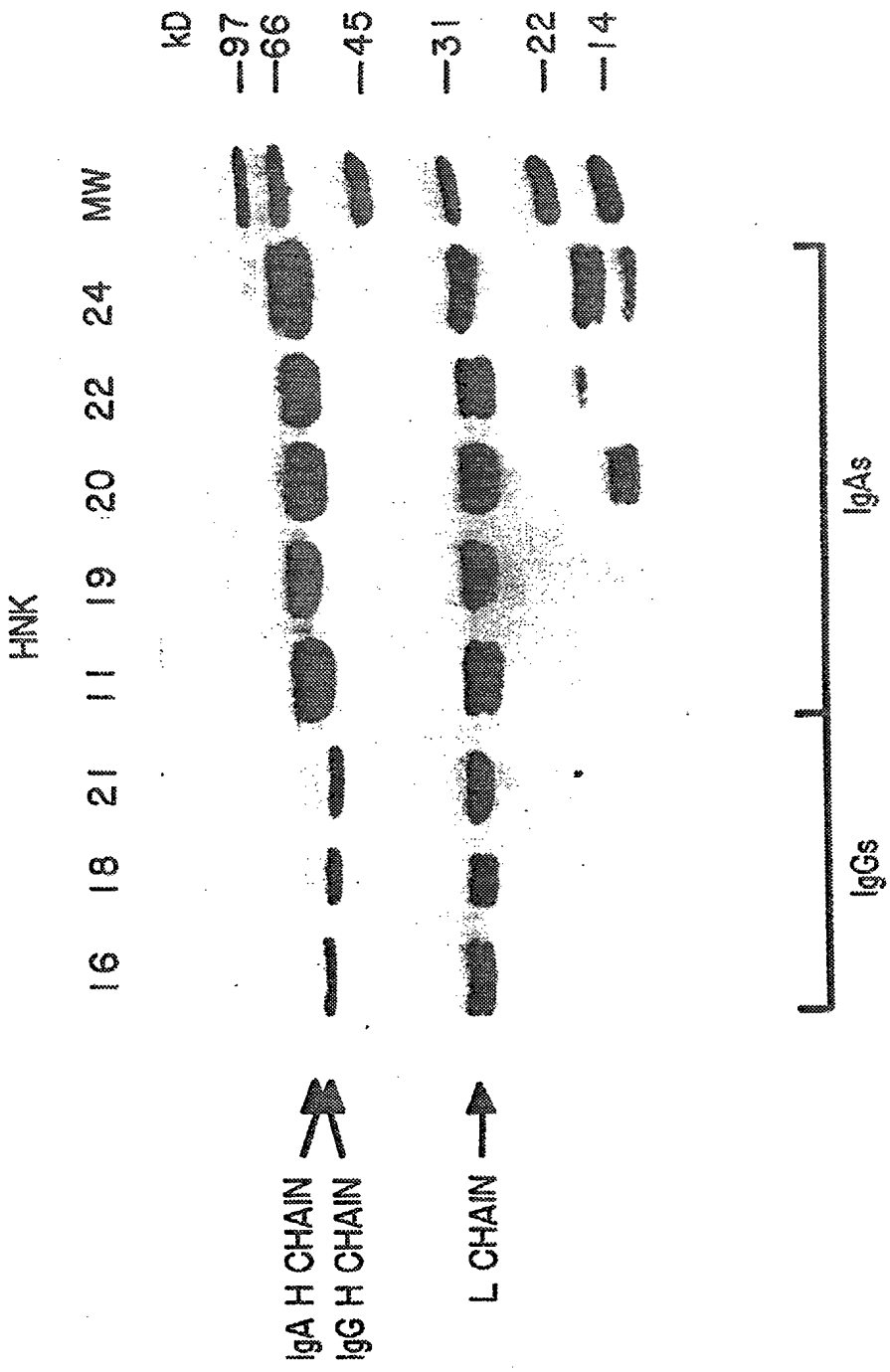
kD
97
66
45
31
22
14
MW
24
22
20
19
11
21
18
16
HNK
IgA H CHAIN
IgG H CHAIN
L CHAIN
IgAs
IgGs

FIG. 1

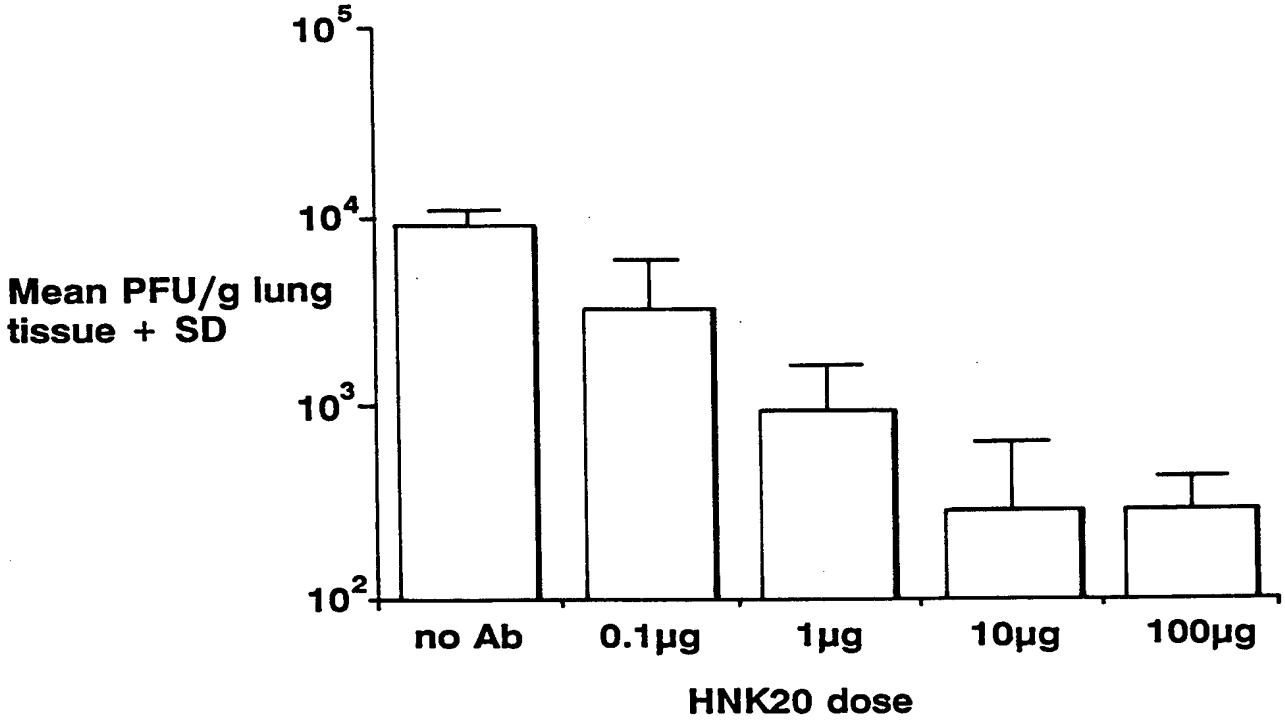
10^5
10^4
10^3
10^2
Mean PFU/g lung tissue + SD
no Ab
0.1µg
1µg
10µg
100µg
HNK20 dose

FIG. 2

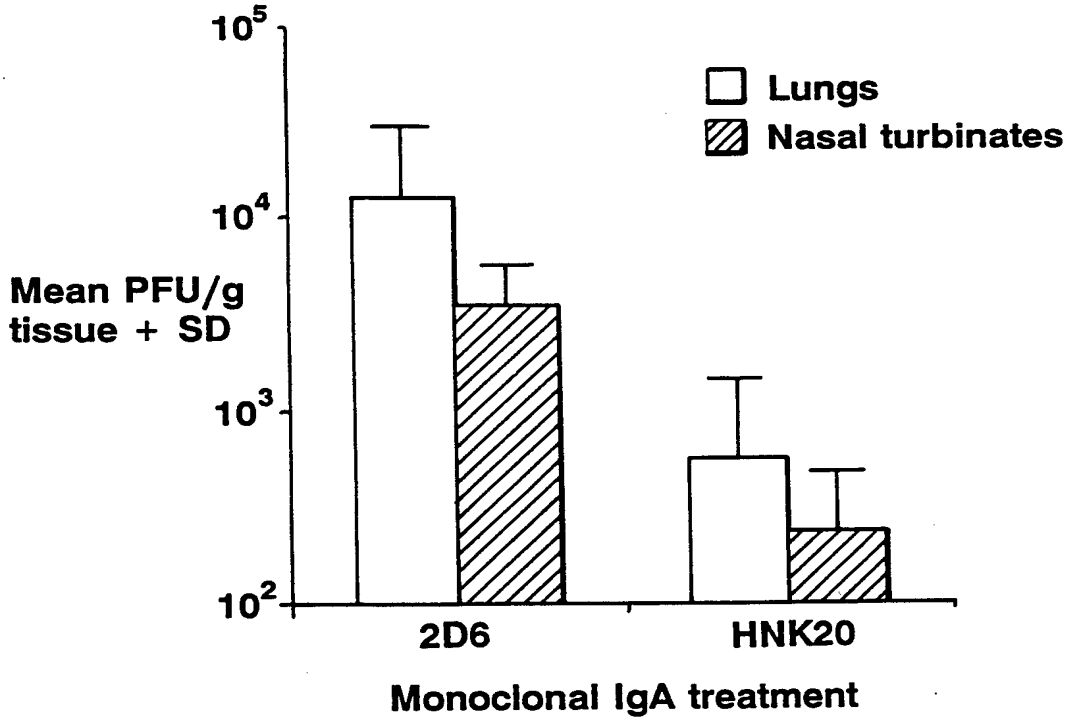
10^5
10^4
10^3
10^2
Mean PFU/g tissue + SD
Lungs
Nasal turbinates
2D6
HNK20
Monoclonal IgA treatment

FIG. 3

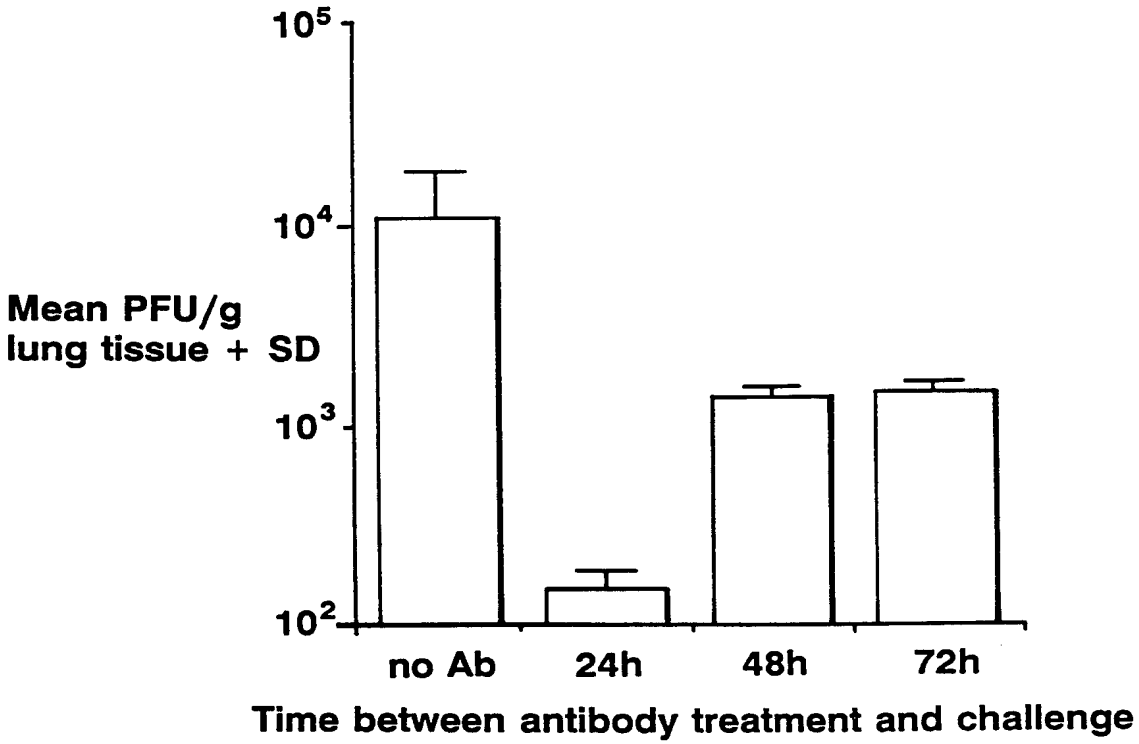
10^5
10^4
10^3
10^2
Mean PFU/g
lung tissue + SD
no Ab
24h
48h
72h
Time between antibody treatment and challenge

FIG. 4

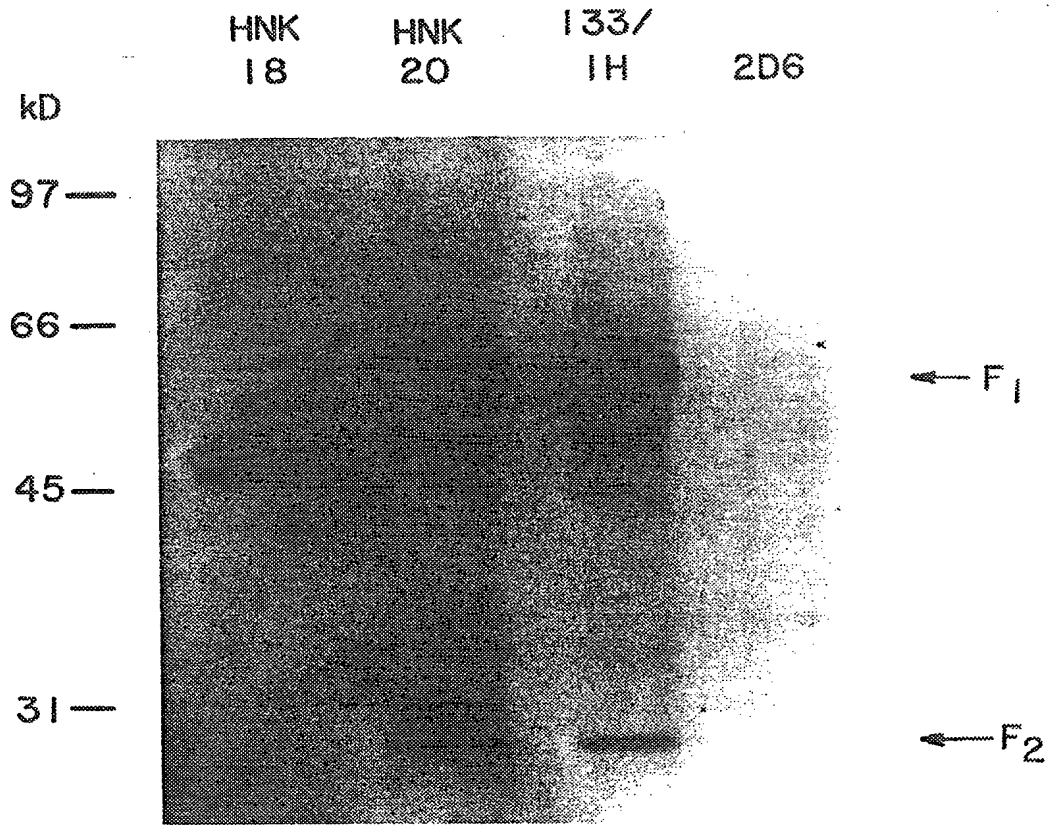

FIG. 5

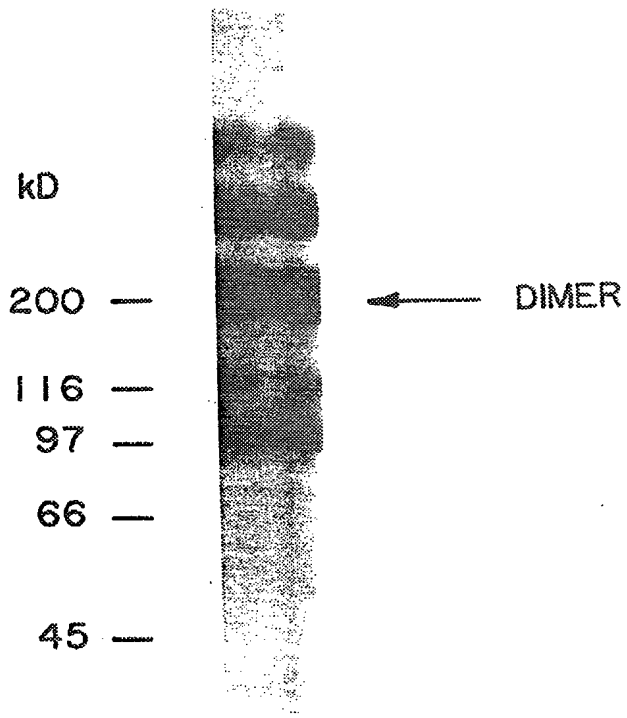

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08699

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 16/08; A61K 39/395; G01N 33/53; C07K 1/00; C12N 5/12, 15/02
US CL :530/388.3, 413; 424/147.1; 435/7.21, 172.2, 240.27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/388.3, 413; 424/147.1; 435/7.21, 70.21, 172.2, 240.27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, DIALOG, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	INFECTION AND IMMUNITY, VOLUME 59, NUMBER 3, ISSUED MARCH 1991, WINNER III ET AL., "NEW MODEL FOR ANALYSIS OF MUCOSAL IMMUNITY: INTESTINAL SECRETION OF SPECIFIC MONOCLONAL IMMUNOGLOBULIN A FROM HYBRIDOMA TUMORS PROTECTS AGAINST *VIBRIO CHOLERAE* INFECTION", PAGES 977-982, SEE PAGES 977-978.	1-17
Y	THE JOURNAL OF CELL BIOLOGY, VOLUME 108, NUMBER 5, ISSUED MAY 1989, WELTZIN ET AL., "BINDING AND TRANSEPITHELIAL TRANSPORT OF IMMUNOGLOBULINS BY INTESTINAL M CELLS: DEMONSTRATION USING MONOCLONAL IGA ANTIBODIES AGAINST ENTERIC VIRAL PROTEINS", PAGES 1673-1685, SEE PAGES 1674-1675.	1-17

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 AUGUST 1994	Date of mailing of the international search report 16 NOV 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PAULA HUTZELL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08699

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF GENERAL VIROLOGY, VOLUME 73, ISSUED 1992, TAYLOR ET AL., "PROTECTIVE EPITOPES ON THE FUSION PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS RECOGNIZED BY MURINE AND BOVINE MONOCLONAL ANTIBODIES", PAGES 2217-2223, SEE PAGE 2217.	1-17
Y	DEVELOP. BIOL. STANDARD, VOLUME 57, ISSUED 1984, STOTT ET AL., "THE CHARACTERIZATION AND USES OF MONOCLONAL ANTIBODIES TO RESPIRATORY SYNCYTIAL VIRUS", PAGES 237-244, SEE PAGE 239.	1-17
Y	JOURNAL OF CELLULAR BIOCHEMISTRY, SUPPLEMENT 15E, ISSUED 1991, JOHNSON ET AL., "DEVELOPMENT OF HUMANIZED MONOCLONAL ANTIBODIES WHICH NEUTRALIZE RESPIRATORY SYNCYTIAL VIRUS", PAGE 120, ABSTRACT NO. N 108, SEE ENTIRE ABSTRACT.	1-17
Y	BRITISH MEDICAL JOURNAL, VOLUME 2, NUMBER 6030, ISSUED 31 JULY 1976, DOWNHAM ET AL., "BREAST-FEEDING PROTECTS AGAINST RESPIRATORY SYNCYTIAL VIRUS INFECTIONS", PAGES 274-276, SEE PAGE 274.	1-17
Y	JOURNAL OF MEDICAL VIROLOGY, VOLUME 17, ISSUED 1985, SCOTT ET AL., "CELLULAR REACTIVITY TO RESPIRATORY SYNCYTIAL VIRUS IN HUMAN COLOSTRUM AND BREAST MILK", PAGES 83-93, SEE PAGE 83.	1-17
Y	CLINICAL CHEMISTRY, VOLUME 27, NUMBER 11, ISSUED 1981, SEVIER ET AL, "MONOCLONAL ANTIBODIES IN CLINICAL IMMUNOLOGY" PAGES 1797-1806, SEE PAGE 1800.	4-8, 13, 14, 15